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Date _____ Sandi Duncan

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Bangur et al.
Application No.	:	09/849,626
Filed	:	May 3, 2001
For	:	COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF LUNG CANCER
Examiner	:	J. Fredman
Art Unit	:	1637
Docket No.	:	210121.478C16
Date	:	

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D. C. 20231

DECLARATION OF CHAITANYA S. BANGUR, Ph.D.

The undersigned, Dr. Chaitanya S. Bangur, hereby declares:

1. I am a Scientist at Corixa Corporation, the assignee of the subject application, and a named inventor of the currently claimed invention. The following experiments were performed under my supervision.

2. Real-time PCR expression analysis was performed for L978P (SEQ ID NO: 1797) in order to further confirm its expression profile in various tumor and normal tissues, including normal lung tissue. This analysis was performed on 87 tumor samples, including sample types comprising primary lung tumors, lung tumor cell lines and lung pleural effusion samples for lung tumor sub-types comprising small cell carcinoma, atypical carcinoid, adenocarcinoma, squamous cell carcinoma, adeno-squamous mix carcinoma, large cell carcinoma and bronchioalveolar carcinoma. Briefly,

quantitation of PCR product relies on the few cycles where the amount of DNA amplifies logarithmically from barely above the background to the plateau. Using continuous fluorescence monitoring, the threshold cycle number where DNA amplifies logarithmically is easily determined in each PCR reaction. There are two fluorescence detecting systems. One is based upon a double-strand DNA specific binding dye SYBR Green I dye. The other uses TaqMan probe containing a Reporter dye at the 5' end (FAM) and a Quencher dye at the 3' end (TAMRA) (Perkin Elmer/Applied Biosystems Division, Foster City, CA). Target-specific PCR amplification results in cleavage and release of the Reporter dye from the Quencher-containing probe by the nuclease activity of AmpliTaq Gold™ (Perkin Elmer/Applied Biosystems Division, Foster City, CA). Thus, fluorescence signal generated from released reporter dye is proportional to the amount of PCR product. Both detection methods have been found to generate comparable results. To compare the relative level of gene expression in multiple tissue samples, a panel of cDNAs is constructed using RNA from tissues and/or cell lines, and real-time PCR is performed using gene specific primers to quantify the copy number in each cDNA sample. Each cDNA sample is generally performed in duplicate and each reaction repeated in duplicated plates. The final Real-time PCR result is typically reported as an average of copy number of a gene of interest normalized against internal actin number in each cDNA sample. Real-time PCR reactions may be performed on a GeneAmp 5700 Detector using SYBR Green I dye or an ABI PRISM 7700 Detector using the TaqMan probe (Perkin Elmer/Applied Biosystems Division, Foster City, CA).

From this analysis, as set forth in the table below, 60/87 tumor samples demonstrated greater than 3-fold over-expression of SEQ ID NO: 1797 relative to normal lung tissue, with 32 of the 60 samples having greater than 10-fold over-expression relative to normal lung tissue.

Lung Cancer		No. Samples	Expression by Real Time PCR	
			> 10-fold over Normal Lung	3 to 10-fold over Normal Lung
Small cell lung carcinoma	primary tumors	2	2	
Small cell lung carcinoma	cell lines	11	6	5

Atypical carcinoid	mets	1	1	
Adenocarcinoma	primary tumors	21	6	7
Adenocarcinoma	LPE	4		1
Adenocarcinoma	cell lines	8		4
Squamous cell carcinoma	primary tumors	23	13	8
Squamous cell carcinoma	cell lines	5	1	1
Adeno-Squamous mix carcinoma	primary tumors	2	2	
Adeno-Squamous mix carcinoma	cell lines	3		
Large cell carcinoma	primary tumors	2		1
Large cell carcinoma	cell lines	4	1	
Bronchioalveolar carcinoma	cell lines	1		1

These results further confirm that SEQ ID NO: 1797 is over-expressed in a high percentage of lung tumor samples of various sub-types relative to normal lung tissue and thereby further confirm the efficacy of SEQ ID NO: 1797 as a sequence effective for identifying the presence of lung cancer in biological samples.

3. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Chaitanya S. Bangur, Ph.D.

4/3/03
Date